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(54) Title: MAGNETIC PARTICLE COMPOSITION

(57) Abstract: A magnetic particle composition which can be used for separating biomolecules comprises magnetic materials of preferably less than five micron diameter bound to a negatively charged ion exchanger. The composition can be used to separate DNA from mixtures and to separate out cell debris.

Magnetic particle composition

The present invention relates to a method of making magnetic and paramagnetic particles which can be used in the separation of biomolecules or compounds from laboratory to industrial scale volumes.

In order to separate biomolecules such as nucleic acids from mixtures containing them it is known to use magnetic particles which are coated with a coating which will attach to the biomolecule which is to be separated. The particles are then added to the mixture and the particles attached to the biomolecules can be separated by use of a magnetic field.

The magnetic particles or beads must be of a size which enables them to remain in suspension in the liquids used. However if they are too small or contain fines or other similar particles they can stay in suspension and can be too slow to separate.

Known methods of making magnetic and paramagnetic particles include :-

1) Incorporation of magnetite (iron oxide, Fe_3O_4) inside porous agarose or cellulose followed by grinding or sieving to obtain a range of particle diameters, usually 1 to 10 microns.
2) Incorporation of magnetite inside silica followed by grinding or sieving to obtain a range of particle diameters, usually 1 to 10 microns.
3) Production of magnetite (less than 10 microns) by precipitation of iron salts, followed by surface coating with a silane or other functional group.
4) Coating a mono-disperse polystyrene bead (less than 10 microns) with sub-micron iron oxide, followed by another coating of polystyrene with or without functional groups.
5) Internal precipitation of iron oxide into a mono disperse polystyrene bead (less than 10 microns) followed by surface coating with functional groups.

Typically, magnetic beads have a diameter of less than 10 microns otherwise their sedimentation rates are too high under gravity for easy handling and the surface area too low to bind desired amount of the target molecule. Some larger beads such as agarose-magnetite may be up to 100microns but they rely on being very porous to bind target molecules internally and separate out under gravity extremely quickly.

Patents US 4,695,392 and US 5,091,206, WO 96/18731, EP 515484B1 disclose methods of forming and using such magnetic particles.

I have devised improved magnetic particles and a method of making them.

According to the invention there is provided a magnetic particle composition which comprises a magnetic material combined with negatively charged ion exchanger.

The invention also provides a method of forming a magnetic particle composition which method comprises contacting a magnetic material with a negatively charged ion exchanger.

The magnetic materials preferably have a diameter of less than ten microns and more preferably five microns or less.

The magnetic material can be any of the conventionally used magnetic materials such as magnetite, iron oxides, transition metal oxides or any ferro or paramagnetic material.

The ion exchanger can be porous or non porous and ion exchangers which can be used include polymethacrylate carboxy ion-exchangers, silica particles coated with a negative charge, cellulose or agarose with phosphate or sulphate groups or any negatively charged species.

The ion exchanger can be attached directly to the magnetic material e.g. by charge alone or it can be attached using a binding agent such as polymerised acrylic acid, or any agent that forms a coating or surface coat to aid cohesion of the materials. The particle may be further derivatised with functional groups such as carboxy, amine, imidazole etc.

The composition can readily be formed by mixing the components in powder form or by pre-mixing in aqueous or non-aqueous solutions with or without the binding agent.

The compositions of the invention i.e. the magnetic material combined with the ion exchanger, preferably have a diameter of between 0.5 microns to 1 mm and more preferably of 20 to 150 microns in diameter.

The ratio of magnetic particle to ion exchanger is not critical and can be varied in accordance with the application, typical ratios are from 5 to 50% (w/w) iron oxide.

Preferably a suspension of the particle composition of the invention in a liquid containing the material to be separated can be easily handled in conventional fluid handling and dispensing systems.

It is a feature of the present invention that the particle compositions provide faster magnetic separation or sedimentation under gravity in larger volumes without residual fines or particles remaining in suspension. The larger particles e.g 20 to 150 microns, also remain in suspension for longer compared to other magnetic beads of similar size made from other materials thus retaining effective mixing and binding kinetics.

The particles can be kept in suspension for dispensing with minimal agitation and also are preferably able to pass through standard pipette tips.

The compositions of the present invention can be used to separate biomolecules from mixtures containing them, for example they can be used to separate nucleic acids and to purify solutions or suspensions by removing impurities such as cell debris etc.

When the compositions are to be used to separate a biomolecule from a liquid containing the biomolecule the particle composition is contacted with a binding agent for the biomolecule for example biotinylated biomolecules may be isolated using Streptavidin coated magnetic particles.

The invention also provides a method for separating biomolecules from mixtures containing them which method comprises contacting a liquid suspension of the biomolecule with the magnetic particle composition as described above to form a suspension of the magnetic particle composition in which the particle composition binds to the biomolecule and applying a magnetic field to the suspension to separate out the magnetic particles having the biomolecule bound thereto.

The biomolecule can be separated from the magnetic particles by conventional means.

The method of the invention can be used to remove cellular debris or insoluble material without centrifugation or filtration.

For example, in the removal of cellular debris from a microbial, plasmid or plant DNA extraction, the particles can be used to rapidly remove unwanted contaminants leaving the target DNA in solution.

The invention is further described in the following examples.

Example 1

6 grams of a granular, porous polymethacrylate carboxy ion-exchanger (100-500 mesh) was mixed with 2 grams of magnetite (Fe_3O_4) of 5 microns diameter or less. This material was then washed in 2% Tween 20 with 1M sodium chloride and used to extract DNA from blood for example: 100mg of the magnetic beads described above were mixed with 1ml of whole blood pre-diluted in 10mM Ammonium Bicarbonate, 1% Tween 20, pH 9. The bound DNA was washed free of contaminants with water and eluted using 10mM Tris pH 9 at 80C.

Example 2

6 grams of a granular, porous polymethacrylate carboxy ion-exchanger (100-500 mesh) was mixed with 2 grams of magnetite (Fe_3O_4) of less than 5 microns diameter This material was then mixed with 50ml of 2% v/v acrylic acid, 2% Ammonium persulphate and heated to 70°C for 30 minutes. This material was then washed in detergents and salts and used to extract DNA from blood as described above.

Example 3

6 grams of a granular, porous polymethacrylate carboxy ion-exchanger (100 - 500 mesh) was mixed with 2 grams of magnetite (Fe_3O_4 less than 5 microns). This material was then mixed with 50ml of 2% v/v acrylic acid, 2% Ammonium persulphate plus 0.02% divinyl benzene and heated to 70°C for 30 minutes.

Example 4

An overnight culture of E.coli was prepared containing a high copy number plasmid. 1 ml of this culture was adjusted to 0.1 M NaOH with 1 % SDS and mixed gently for 5 minutes. Then the magnetic particles as described in Example 1 were added and the suspension adjusted to 1 M potassium acetate pH 5.5. Following magnetic separation all the insoluble debris was removed in less than 1 minute leaving a clarified supernatant containing plasmid DNA ready for further processing.

Claims

1. A magnetic particle composition which comprises a magnetic material combined with negatively charged ion exchanger.

2. A composition as claimed in claim 1 in which the magnetic materials have a diameter of less than ten microns.

3. A composition as claimed in claim 1 in which the magnetic materials have a diameter of less than five microns.

4. A composition as claimed in any one of claims 1 to 3 in which the magnetic materials have a diameter of less than 1 micron.

5. A composition as claimed in any one of claims 1 to 4 in which the ion exchanger is porous or non porous.

6. A composition as claimed in claim 1 to 5 in which the ion exchanger is a polymethacrylate carboxy ion-exchangers, or cation exhanger.

7. A composition as claimed in any one of claims 1 to 6 in which an ion exchanger is attached directly to the magnetic material.

8. A composition as claimed in any one of claims 1 to 6 in which the ion exchanger is attached to the magnetic materials by a binding agent.

9. A composition as claimed in 8 in which the binder is selected from monomers or polymers of acrylic acid, acrolein, amines, amides, alcohols, aldehydes, organic acids, imidazoles, phosphates, secondary, tertiary or quaternary amines or sulphates.

10. Compositions as claimed in any one of claims 1 to 9 which have a diameter of between 0.5 microns to 1 mm

11. Compositions as claimed in any one of claims 1 to 9 which have a diameter of between 20 to 150 microns in diameter.

12. A compositions as claimed in any one of claims 1 to 9 in which the ratio of magnetic material to ion exchanger is from 0.05 to 2.

13. A method of forming a magnetic particle composition which mixing a powder of a magnetic material with a powder of a negatively charged ion exchanger.

14. A method as claimed in claim 13 in which the magnetic particles have a diameter of less than ten microns.

15. A method as claimed in claim 13 in which the magnetic particles have a diameter of less than five microns.

16. A method as claimed in any one of claims 13 to 15 in which the magnetic particles are 10 to 150 microns.

17. A method as claimed in any one of claims 13 to 16 in which the ion exchanger is porous or non porous.

18. A method as claimed in claim 13 to 17 in which the ion exchanger is a polymethacrylate carboxy ion-exchanger, or cation exchanger.

19. A method as claimed in any one of claims 13 to 18 in which the ion exchanger is attached directly to the magnetic material.

20. A method as claimed in any one of claims 13 to 18 in which the ion exchanger is attached to the magnetic materials by a binding agent.

21. A method as claimed in 20 in which the binder is monomers or polymers of acrylic acid, acrolein, amines, amides, alcohols, aldehydes, organic acids, imidazoles, phosphates, secondary, tertiary or quaternary amines or sulphates

22. A method as claimed in any one of claims 13 to 21 in which the ratio of magnetic material to ion exchanger is from 0.05 to 2.

23. A magnetic particle composition made by the method of any one of claims 13 to 22.

24. A method for separating biomolecules from mixtures containing them which method comprises contacting a liquid suspension of the biomolecule with the magnetic particle composition as claimed in any one of claims 1 to 12 or 23 to form a suspension of the magnetic particle composition in which the particle composition binds to the biomolecule and applying a magnetic field to the suspension to separate out the magnetic particles having the biomolecule bound thereto.

INTERNATIONAL SEARCH REPORT

Inter ɔnal Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 H01F1/11 H01F1/44 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 H01F C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 196 24 426 A (BERGEMANN CHRISTIAN) 2 January 1998 (1998-01-02) column 4, line 20 -column 5, line 44 column 6, line 30 - line 48; claims 1,4,5,10,23	1-10, 13-15, 17-21, 23,24
X	US 5 091 206 A (SHAH DINESH O ET AL) 25 February 1992 (1992-02-25) cited in the application column 4, line 40 - line 51; claims 1,2,4,5	1-8,12, 13, 17-20, 23,24

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

information on patent family members

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